REMARKS

Applicants respectfully request that the amendments presented in the Amendment and Response under 37 C.F.R. § 1.116, filed August 13, 2003 not be entered.

Claims 15-18 and 28-30 are pending in the application. In order to reintroduce additional dependent claims and incorporate the amendments proposed but not entered in the Amendment and Response under 37 C.F.R. § 1.116, filed August 13, 2003, the claims have generally been rewritten and amended as follows:

New claim 31 generally corresponds to previous claim 16. New claim 37 generally corresponds to previously cancelled claim 16 and has been amended at step (b) to refer to "a second layer of cells comprising epithelial cells disposed on the first layer." Support for this amendment can be found in original claim 9 and previous claim 15. New claim 39 generally corresponds to previous claim 15. New claim 48 generally corresponds to previous claim 29. New claims 50-52 generally correspond to previous claim 28, but this claim has been separated into three independent claims based on new independent claims 31, 37 and 39 discussed above.

With regard to the dependent claims, new claims 32 and 43 generally correspond to previously cancelled claim 6. New claims 33 and 42 generally correspond to previously cancelled claim 14. New claims 34 and 44 generally correspond to previous claim 17. New claims 35 and 45 generally correspond to previous claim 18. New claims 36 and 47 generally correspond to previous claim 30. New claims 38 and 46 generally correspond to previously cancelled claim 10. New claims 40 and 49 generally correspond to a combination of the elements in previously cancelled claims 11 and 23. New claim 41 generally corresponds to previously cancelled claim 12.

Amendments have also been made to correct typographical errors and to better define the claimed invention.

Accordingly, no new matter has been introduced by these amendments and new claims.

Therefore, after entry of this amendment, claims 31-52 will be pending in this application.

The outstanding rejections are addressed individually below.

The Advisory Action states that the amendment of claims 15 and 16 to recite the claim limitation "first layer of cultured fibroblasts cells which produce a layer of extracellular matrix" would require further consideration under 35 U.S.C. §112, first paragraph, regarding enablement issues. Applicants respectfully submit that this claim limitation is enabled by the specification as filed.

The specification of the instant application clearly states that, "[t]he predominant major extracellular matrix component produced by fibroblasts is fibrillar collagen, particularly collagen type I" and continues to state that

> other collagens, both fibrillar and non-fibrillar collagen from the collagen family such as collagen types II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XII, XIV, XV, XVI, XVII, XVIII, XIX, may be produced by use of the appropriate cell type. Similarly, other matrix proteins which can be produced and deposited using the current method include, but are not limited to elastin; proteoglycans such as decorin or biglycan; or glycoproteins such as tenascin; vitronectin; fibronectin; laminin, thrombospondin I, and glycosaminoglycans (GAG) such as hyaluronic acid (HA). (Page 8, lines 20-29)

Various sources of fibroblast cell strains used in the invention are disclosed at page 7, lines 4-19. Suitable vessels and growth surfaces for the culture of the fibroblast cells are described at page 9, line 1 to page 10, line 20. Culture media formulations for culturing the fibroblasts are disclosed at page 11, line 11 to page 17, line 24, and environmental conditions for their culture are described at page 17, lines 25-28. A description of seeding and culturing the fibroblasts in order to obtain a layer of cultured fibroblasts and extracellular matrix is described at page 17, line 29 through page 18, line 26. The specification states that "[w]hen fully formed, the constructs of the invention have bulk thickness due to the fibrous matrix produced and organized by the cells . . . ." (Page 18, lines 26-28) In the fabrication of the cultured tissue construct of the invention,

the cells will form an organized matrix around themselves on the cell culture surface. This extracellular matrix layer, with the cultured fibroblast cells contained within it, has a measurable thickness. (Page 19, lines 1-6) This layer has cohesive properties, due primarily to the bulk thickness and fibrous matrix structure, and is handleable in that it can be manually peeled from the culture substrate such that it may be applied to a patient in a clinical setting. (Page 23, line 26 to page 24, line 6)

Further, Applicants have shown, in several of the Examples, a first layer of cultured fibroblast cells that produce an extracellular matrix layer. Example 1 describes the formation of a collagenous matrix formed by human neonatal foreskin fibroblasts. Example 3 describes the *in vitro* formation of a collagenous matrix by human neonatal foreskin fibroblasts in chemically defined medium. Example 5 describes the *in vitro* formation of a collagenous matrix formed by human Achilles tendon fibroblasts. Example 6 describes the *in vitro* formation of a collagenous matrix formed by transfected human neonatal foreskin fibroblasts. Example 9 describes the *in vitro* formation of a matrix formed by human corneal keratocytes. Example 10 describes the *in vitro* formation of a collagenous matrix formed by human neonatal foreskin fibroblasts seeded in production media. Example 11 describes the *in vitro* formation of a collagenous matrix formed by pig dermal fibroblasts. Example 15 describes the *in vitro* formation of three collagenous matrices formed by human neonatal foreskin fibroblasts in three differently supplemented chemically defined media. Example 17 describes the formation of a collagenous matrix formed by human buccal fibroblasts. The extracellular matrix layer has measurable physical and mechanical properties as shown in Example 14 where the cell-matrix constructs produced in Examples 1 (cell-matrix construct), 2 (cell-matrix construct with a keratinocyte layer thereon) and 3 (cell-matrix construct formed in defined medium) are evaluated.

Therefore, Applicants respectfully submit that the claim limitation of "a first layer of cultured fibroblasts cells that produce a layer of extracellular matrix" is enabled by the specification as filed.

Applicants note that the Advisory Action also indicates that the scope of the invention as claimed encompasses fibroblasts, which endogenously produce extracellular matrix, comprising: (i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern; (ii) decorin; (iii) fibronectin; (iv) tenascin; and (v) glycosaminoglycans. (Applicants assume that these are the components of the extracellular matrix that were intended to be listed as these are the components listed in previous claim 16). The Advisory Action also states that the specification as filed fails to disclose the synthesis of the above-mentioned extracellular components by cultured fibroblasts, wherein the fibroblasts have not been genetically modified to produce the extracellular matrix components. Applicants respectfully disagree.

The specification of the instant application discusses one preferred embodiment comprising a structural layer of at least one type of extracellular matrix-producing cells and endogenously produced extracellular matrix components wherein the matrix is completely cell-synthesized and assembled by culturing the cells. Some biochemical features detected in the matrix are listed, including: (i) collagen types I and III exhibiting the quarter staggered 67 nm banding pattern; (ii) decorin; (iii) fibronectin; (iv) tenascin; and (v) glycosaminoglycans. (See page 4, line 1 to page 5, line 2. See also page 8, lines 20-29, discussed above.) Fibroblast cells, generally, produce a number of extracellular matrix proteins, primarily collagen. (Page 7, lines 2-3) Therefore, fibroblasts used to produce extracellular matrix with these features are normal and need not be genetically modified.

While Applicants have shown that genetically modified cells are not required to produce the claimed extracellular components in an extracellular matrix layer, recombinant cells or genetically engineered cells may also be used in this invention. (See page 7, line 28 to page 8, line 19) For instance, Example 6 illustrates the *in vitro* formation of a collagenous matrix by transfected human neonatal foreskin fibroblasts where the transfected cells produce platelet-derived growth factor (PDGF). Samples of the resultant cell-matrix constructs displayed a similar gross appearance to the

constructs produced in Example 1 using normal cells but also exhibited PDGF output measuring 100 ng/mL by ELISA assay throughout the culture (18 days) while PDGF output by control constructs was undetectable. (See page 33, line 22 to page 35, line 3)

Therefore, Applicants have shown that both non-genetically modified fibroblasts and genetically modified cells may be used to produce the claimed invention.

CONCLUSIONS

In view of the arguments set forth above, Applicants respectfully submit that the rejections contained in the Advisory Action mailed on September 30, 2003, have been overcome, and that the claims are in condition for allowance. If the Examiner believes that any further discussion of this communication would be helpful, she is invited to contact the undersigned at the telephone number provided below.

Applicants enclose herewith a petition for a one month extension of time pursuant to 37 C.F.R. § 1.136, up to and including November 15, 2003 (based on the Notice of Appeal received by the USPTO on August 15, 2003). Please charge deposit account no. 08-0219 the $55.00 fee for this purpose.

Applicants also herein request continued examination of the application according to 37 C.F.R. § 1.114. Please charge the same deposit account the $385.00 fee set forth in 37 C.F.R. § 1.17(e) for this purpose.

No other fees are believed to be due in connection with this response. However, please charge any underpayments or credit any overpayments to Deposit Account No. 08-0219.

Respectfully submitted,

Alison E. Corkery

Alison E. Corkery
Reg. No. 52,770

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HALE AND DORR LLP
60 State Street
Boston, MA 02109
Tel: (617) 526-6000
Fax: (617) 526-5000